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VACCINATION OF SWINE AGAINST TUBERCULOSIS WITH CALMETTE-GUÉRIN CULTURE, BCG¹

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INTRODUCTION

Swine being highly susceptible to bovine tuberculosis and, under many systems of swine husbandry, exposed to infectious material from tuberculosis cattle, it was thought advisable to carry out certain experiments at the University of California to test the protective effect of the Calmette-Guérin culture known as BCG, upon hogs under controlled exposures to infection. Results of similar experiments conducted with cattle have already been published by the writers.⁽⁵⁾

REVIEW OF LITERATURE

At the time the experiments reported herein were started no publications were known to the writers regarding the immunizing effect of BCG on swine, and contributions appearing since that time are not in agreement.

Ascoli^(1, 2) and his collaborators reported an experiment with 12 pigs, 6 of which were vaccinated before the tenth day of age. The other 6 were retained unvaccinated as controls. Of the 6 vaccinated pigs, 3 were given the vaccine by mouth, each receiving a dose of 10 mg on three alternate days. The other 3 pigs were vaccinated by injecting each sub-

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cutaneously behind the ear with one dose of 10 mg. In 1 of these latter (No. 13) the effect was considered unsatisfactory by Ascoli because it showed sloughing at the point of injection. The test infection was given twelve weeks later by injecting into the ear vein a suspension of virulent bovine tubercle bacilli. Autopsy notes were published only for 3 of the vaccinated pigs and 1 of the controls. These 4 cases, in which each received 1 mg of virulent bacilli intravenously August 5, 1926, may be summarized as follows:

Pig No.	Vaccinated	Date slaughtered	Tuberculous lesions
19	Subcutaneously 10 mg on May 15, 1926	March 10, 1927	Slight in bronchial and mediastinal lymph nodes
13	Subcutaneously 10 mg on May 11, 1926	June 30, 1927	Tubercles marked in bronchial, mesenteric, hepatic and pharyngeal lymph nodes and in liver; slight in kidney
2	By mouth 10 mg doses on May 11, 13, and 15, 1926	June 30, 1927	Moderate lung lesions, extensive lymphatic lesions; more extensive liver lesions than No. 13
4	Control	June 22, 1927	Extensive in lungs and lymphatics

Four of the other pigs had received 0.02 mg virulent tubercle bacilli intravenously and 4 only 0.0005 mg. Ascoli did not state what was found when these 8 swine were butchered except that No. 20 which had been vaccinated subcutaneously and then infected intravenously with 0.0005 mg came nearest to the degree of resistance exhibited in No. 19. He concluded that vaccination by mouth had failed to develop any perceptible resistance to the particular kind of test infection which he had used. On the other hand he stated that the results in the swine vaccinated subcutaneously justified the conclusion that this method is capable of "pre-munizing" swine against tuberculous infection.

From these two publications by Ascoli, the writers can not see how he and his coworkers are justified in such a conclusion, particularly since this was apparently based on the relatively slight lesions found in his swine No. 19. It should be noted that this swine was slaughtered 104 days before the control and it seems to the writers that if it had been permitted to live until June 30, 1927, the lesions might have developed to the extent of those found in control No. 4 or the pig (No. 2) which had been vaccinated by mouth.

Sanz⁽⁸⁾ in Chile has reported the vaccination of 993 pigs, but stated he had never seen any signs of tuberculosis following vaccination. Most

of his experiments were apparently field trials without the use of unvaccinated controls. However, he states that in herds of swine badly infected with tuberculosis through the ingestion of milk from tuberculous cows, the systematic vaccination of all newborn pigs caused an arrest of the tuberculous infection while the older swine not vaccinated continued to die of tuberculosis.

Jundell and Magnusson⁽⁷⁾ carried out an experiment with 24 pigs. Eight of them were vaccinated when 14 to 16 days old by injecting 10 mg BCG subcutaneously near the point of the breast bone; eight others when 3 to 9 days old were given 10 mg doses by mouth on three alternate days; and eight were retained as controls.

After two months the 16 vaccinated pigs and 6 of the controls were subjected to infection by feeding to each animal sweet milk mixed with 50 cc of a thick puree obtained by grinding udder lesions of a tuberculous cow. It was estimated that each pig received about 2 billion tubercle bacilli. The 2 controls which were not fed this material were retained on the same premises.

In 19 to 22 weeks the entire 24 swine were slaughtered and all found to be tuberculous except the 2 controls which had not eaten the tuberculous udder tissue. Meat inspectors condemned as unfit for food 3 of the controls, 4 of those vaccinated by mouth, and 4 of those vaccinated subcutaneously. The investigators stated that scarcely a perceptible difference existed in the extent of tuberculosis between the control and the vaccinated animals and no indications of protection against tuberculosis in swine were attributable to the vaccine. However, their experiments on calves with BCG vaccine prepared in a similar way to that used on the pigs had exercised a preventive action against tuberculosis in 50 per cent of the calves. The pigs vaccinated subcutaneously developed an induration at the point of injection which was distinct at the end of the first month, but had completely disappeared by the end of the second month. At the time of slaughter no lesions were visible at the inoculation points. From this they concluded since none of the 8 pigs vaccinated subcutaneously had developed an abscess, that swine in general do not have so acute a local reaction toward BCG as do larger animals.

Also according to Jundell and Magnusson an experiment by Jerlov⁽⁸⁾ was made August 31, 1926, by injecting a 14-day-old pig subcutaneously with 50 mg BCG; a pig of similar age served as a control. Six days after vaccination, tumefaction at the point of inoculation had disappeared, and no reaction was observed afterwards at this point. On September 23 the tuberculin test by the intracutaneous method gave negative results in both animals, but on October 9 the vaccinated animal gave a manifest

reaction to tuberculin, while the control was still negative. Beginning October 17 both animals were fed at six different times with fragments of lungs from a tuberculous cow. After a time they began to lose weight, especially the vaccinated pig. They were slaughtered at the age of six months, and both presented very advanced and generalized tuberculosis. In the vaccinated animal the lesions had especially a progressive character and the lymphatic glands were fused, while in the control they were in part calcified. Jerlov considered the infective doses were too massive, and did not therefore wish to draw conclusions.

In laboratory experiments at Utrecht, de Blicke⁽⁴⁾ found that the resistance of the calves was increased by BCG vaccination to a greater extent than that of pigs, but he did not think the vaccination gave a specific immunity.

EXPERIMENTAL METHODS USED

In the main, the experimental procedures with swine reported herewith followed closely those carried out on cattle at the California station,⁽⁵⁾ especially in relation to the technique of preparing and administering the vaccine, forms of exposure to infection, and autopsy methods.

Source of Pigs Used in the Experiments.—The animals used in the experiments were all secured from sources believed to be free from tuberculosis, and in addition were tuberculin-tested by intradermic injection into an ear of 5 per cent solution of precipitated tuberculin in sterile distilled water. In most cases the swine were farrowed by tuberculosis-free sows on noninfected premises of the University campus at Davis and kept free from any contact with tuberculous animals from birth until the time of artificial infection. All were vaccinated against hog cholera with serum and virus at approximately six weeks of age or at a time to allow them to be fully over the effects before exposure to tuberculosis. The pigs vaccinated with BCG were kept separated from those to be used as controls until exposure, when all were allowed to run together.

Preparation of the Vaccine.—The original cultures of BCG used in the experiments were obtained by one of the writers (Traum) at the Pasteur Institute, Paris, on April 7, 1926. Care has been taken to grow and prepare the vaccine and test it for pathogenicity on guinea pigs in exactly the way prescribed in directions received from Calmette.⁽³⁾ The stock cultures have been maintained in Roux tubes on potato with 5 per cent glycerine broth for a series of nine generations. The tenth

and eleventh generations have been propagated on 5 per cent glycerinated ox-bile potato and then replanted on potato in glycerine broth for nine generations. Old, fully-ripened potatoes are used, because it has been observed that media made from new potatoes give only a feeble growth of BCG. Sauton's medium has been used for propagating some of the vaccine serials. A separate incubator planting room and equipment have been used exclusively for BCG culture and vaccine preparation. The vaccine has been made from cultures not less than 19 days nor more than 26 days old. When ready for use, each cubic centimeter of vaccine contained 10 mg of BCG bacilli (weight after removal of excess moisture by blotting with filter paper), suspended in a sterile diluent consisting of 100 parts of distilled water, 1 part of chemically pure glucose, and 1 part of chemically pure glycerine, as prescribed by Calmette.⁽³⁾

Experimental Groups.—The objects of the investigations were to test the value of BCG as an immunizing agent against tuberculosis in swine, the length of time after vaccination that protection, if any, developed, and to determine the best methods and ages for vaccination. There are, therefore, included in the records to be presented the following groups: (1) Fourteen pigs that were subcutaneously vaccinated with 100 mg of BCG at ages varying from 3 days to 5 days and exposed to feeding infection at 27 or 93 days after vaccination, and with an equal number of controls, were killed for examination at intervals varying from 90 to 297 days after the first exposure (tables 2A and 2B). (2) Eight that were subcutaneously vaccinated with 100 mg of BCG at ages varying from 65 days to 171 days and exposed to feeding infection at 18 or 140 days after vaccination, and with 4 controls, were killed for examination from 129 to 176 days after the first exposure (tables 3A and 3B). (3) Five that were intramuscularly vaccinated with 100 mg of BCG, exposed 28 or 34 days thereafter to infection with milk from a cow with tuberculosis of the udder, and with 6 controls, were killed at 36 and 74 days after the first milk feeding (table 4). (4) Six that were subcutaneously vaccinated with 100 mg of BCG at 105 days of age, exposed to infection by intravenous injection of a virulent culture of bovine tuberculosis 60 days thereafter, and with 5 controls, were killed from 237 to 243 days after injection of the culture (table 5). (5) Two that were vaccinated intravenously with 1.0 mg of BCG at 93 days of age, exposed to feeding infection 62 days thereafter, and with 3 controls, were killed 165 days after the first exposure (table 6). (6) Four intradermally vaccinated at the ages of 7, 8, and 93 days with 50 mg of BCG, exposed to feeding infection 60 or 62 days thereafter, and with an equal number of

controls, were killed from 165 to 200 days after first exposure (tables 7 and 8). (7) Six which were given three doses of BCG by mouth at the age of 7 and 8 days, exposed to feeding infection 60 days thereafter, and with 5 controls, were killed between 185 and 200 days after first exposure (table 9).

Autopsy Methods and Guinea Pig Injections.—All of the swine were slaughtered in small local slaughter establishments where official inspection was maintained, with the exception of those that showed physical indications of tuberculosis. These were killed in the University laboratories at Davis. In either case all of the organs and the principal body glands were carefully examined for lesions by slicing thin sections. When lesions were present some of those from the head, the thoracic and abdominal cavities, and occasionally from other areas, were removed without slicing for guinea pig inoculation. If no lesions could be seen the apparently normal tissues in these parts were used for guinea pig injections. The usual procedure was to inject guinea pigs with material from head, bronchial, gastrohepatic, and mesenteric glands, and from lungs, spleen, and liver, whether lesions were present or not. Before injection of any tissues removed from the organs, or from any lymph nodes, the tissues were first immersed in boiling water from 10 to 12 seconds and carefully and thinly sectioned with sterilized scissors for observation of any small lesions in the apparently healthy tissues. These sections were ground in sterile mortars with physiological sodium chloride solution, examined by smears for acid-fast bacteria and from 1.0 to 2.0 cc injected intramuscularly into guinea pigs. The guinea pigs were usually killed between 60 and 90 days after inoculation.

SUBCUTANEOUS VACCINATION AND INFECTION EXPOSURE BY FEEDING

Method of Vaccination.—All of the swine in this group received 100 mg of BCG vaccine suspended in 10 cc of diluent, the preparation of which has been previously described. The injections were made with an 18-gauge hypodermic needle into the subcutaneous fascia of the flank. In all animals, except pig No. 9 (table 2A), 5 cc were injected in each flank; No. 9 had the entire 10 cc (100 mg) introduced into the left flank.

Character of Infectious Material and Method of Feeding.—The tissues fed were obtained from tuberculous cattle condemned at an abattoir or from guinea pigs, rabbits, and hogs infected with bovine tuberculosis. The character of the material, approximate quantity fed, and microscopic and guinea pig tests are outlined in table 1.

TABLE 1

SOURCES AND AMOUNTS OF TUBERCULOUS TISSUE USED TO INFECT SWINE BY FEEDING*

Pig Nos. and number of feedings	Date collected	Description of material	Dates fed	Dose for each pig		Guinea pig control result†
				Grams of tissue‡	Estimated bacilli‡	
1-14 and 101-114 (10 feedings)	Aug. 12 (1927)	Tuberculous tissue from the lungs, costal pleura, and lymph nodes of 2 aged cows and a calf, 7 months old.....	Aug. 13	2.0	500,000	+
			Aug. 15	2.0	500,000	+
			Aug. 17	2.0	500,000	+
	Aug. 18	Composite sample of tuberculous tissues from an aged cow.....	Aug. 19	2.0	Positive smears	+
			Aug. 22	2.0	Positive smears	+
			Aug. 23	2.0	Positive smears	+
	Aug. 26	Tuberculous tissue from 2 rabbits and 2 guinea pigs.....	Aug. 28	1.0	1,320,000	+
			Sept. 2	1.0	1,650,000	+
	Sept. 7	Lung of calf No. 101 which died of milary tuberculosis following intravenous injection of virulent culture....	Sept. 9	0.5	Positive smears	+
			Sept. 10	0.5	Positive smears	+
17-22 and 116-118 (30 feedings)	Aug. 12 (1927)	Tuberculous tissue from the lungs, costal pleura and lymph nodes of 2 aged cows and a calf, 7 months old.....	Aug. 13	10.0	500,000	+
			Aug. 15	10.0	500,000	+
			Aug. 17	2.0	690,000	+
	Aug. 16	Tuberculous tissue from the lungs, costal pleura and lymph nodes of an aged cow.....	Aug. 19	2.0	690,000	+
			Aug. 22	2.0	690,000	+
			Aug. 23	2.0	690,000	+
			Aug. 27	2.0	1,320,000	+
	Aug. 26	Tuberculous tissue from 2 rabbits and 2 guinea pigs.....	Aug. 27	2.0	1,320,000	+
	Sept. 1	Tissues from lungs and lymph nodes of a range steer.....	Sept. 2	2.0	850,000	+
			Sept. 8	0.5	1,890,000	+
	Sept. 7	Lung of calf No. 101 which died of milary tuberculosis.....	Sept. 9	0.5	1,890,000	+
			Sept. 11	2.0	960,000	+
	Sept. 10	Lymph node tissue, calf No. 101.....	Sept. 12	2.0	960,000	+

* Other forms of infection used are described under their respective headings in the text.

† This represents the weight of tuberculous tissue after grinding but before straining through several thicknesses of cheese cloth. The actual amount of original tissue in each dose is less than one-third of this weight.

‡ Made by suspending 10 or 20 grams of thoroughly ground tuberculous tissue in a measured quantity of physiological sodium chloride solution; then estimating according to the technique for the direct microscopic counting of bacteria in milk. The estimate was only comparative. The number of organisms consumed was probably far greater than indicated in this column, since the character of the tissue and the method of estimating did not lend themselves to more definite determination of the number of organisms.

¶ The inoculum for the guinea pigs was a portion of the ground and strained tuberculous materials suspended in milk as fed to the calves and the weight is given in terms of ground but unstrained material.

TABLE 1—(Continued)

Pig Nos. and number of feedings	Date collected	Description of material	Dates fed	Dose for each pig		Guinea pig control result†
				Grams of tissue†	Estimated bacilli‡	
17-22 and 116-118 (30 feed- ings) (Cont'd)	Sept. 13 (1927)	Lung of calf No. 102, which died of mili- ary tuberculosis.....	Sept. 14	5.0	1,950,000	+
			Sept. 16	5.0	1,950,000	+
			Sept. 18	0.3	2,000,000	+
	Sept. 13	Lung and lymph nodes, calf No. 102.....	Sept. 19	0.3	2,000,000	+
	Sept. 23	Tuberculous viscera from guinea pigs that had been inoculated with the feeding material used on Aug. 13, as shown in this table.....	Sept. 24	1.0	2,040,000	+
			Sept. 28	0.5	1,020,000	+
			Sept. 29	0.5	5,850,000	+
			Sept. 30	0.5	5,850,000	+
			Oct. 1	0.5	5,850,000	+
			Oct. 2	0.5	5,850,000	+
			Oct. 3	0.5	5,850,000	+
			Oct. 4	0.5	5,850,000	+
			Oct. 5	1.11	12,980,000	+
			Oct. 6	1.11	12,980,000	+
			Oct. 7	1.11	12,980,000	+
			Oct. 8	1.11	12,980,000	+
	Oct. 10	Tuberculous viscera of 14 guinea pigs inoculated from feedings Sept. 2-8 shown in this table.....	Oct. 11	1.11	12,980,000	+
			Oct. 12	1.11	12,980,000	+
15-16 and 115 (20 feed- ings)	Sept. 23 (1927)	Tuberculous viscera from guinea pigs that had been inoculated with the feeding material used on Aug. 13, as shown in this table.....	Sept. 24	1.0	2,040,000	+
			Sept. 28	0.5	1,020,000	+
			Sept. 29	0.5	5,850,000	+
	Sept. 28	Caseous material from tuberculous le- sions in the lung and lymph nodes of an aged cow.....	Sept. 30	0.5	5,850,000	+
			Oct. 1	0.5	5,850,000	+
			Oct. 2	0.5	5,850,000	+
			Oct. 3	0.5	5,850,000	+
			Oct. 4	0.5	5,850,000	+
			Oct. 5	1.11	12,980,000	+
			Oct. 6	1.11	12,980,000	+
			Oct. 7	1.11	12,980,000	+
			Oct. 8	1.11	12,980,000	+
	Oct. 10	Tuberculous viscera of 14 guinea pigs inoculated from feedings, Sept. 2-8 shown in this table.....	Oct. 11	1.11	12,980,000	+
			Oct. 12	1.11	12,980,000	+
	Oct. 13	Tuberculous viscera of 8 guinea pigs inoculated with bovine lesions.....	Oct. 14	1.8	45,000,000	+
			Oct. 15	1.8	45,000,000	+
	Oct. 15	Tuberculous viscera of 16 guinea pigs inoculated with feedings, Sept. 9-14, shown in this table.....	Oct. 16	4.8	67,500,000	+
			Oct. 17	4.8	67,500,000	+
	Oct. 18	Tuberculous viscera of 21 guinea pigs inoculated with feedings Sept. 14-19, shown in this table.....	Oct. 19	6.8	73,100,000	+
			Oct. 20	11.0	3,500,000,000	+
	Oct. 19	Viscera of 3 guinea pigs and a rabbit which had died of tuberculosis after injection with bovine lesions.....	Oct. 20	11.0	3,500,000,000	+

TABLE 1—(Concluded)

Pig Nos. and number of feedings	Date collected	Description of material	Dates fed	Dose for each pig		Guinea pig control result†
				Grams of tissue‡	Estimated bacilli‡	
34-35, 38-39, and 134-139 (5 feed- ings)	Aug. 24 (1928)	Tuberculous lymph nodes from a cow and a pig obtained from slaughter house.....	Aug. 25	13.2	One acid-fast per 80 fields	+
	Aug. 31	Tuberculous lymph nodes from several dairy cows, killed at local slaughter house.....	Aug. 31	16.0	160,000	+
			Sept. 1	16.0	160,000	+
	Sept. 4	Lungs from a dairy cow killed at a local abattoir, extensive tuberculosis with much caseous material.....	Sept. 5	43.0	154,000,000	+
			Sept. 6	43.0	154,000,000	+
36-37, 40-45, and 140-144 (4 feed- ings)	May 31 (1928)	Tuberculous lesions from control pigs 112 and 113, killed on May 31, 1928.....	June 1	17.0	Positive smears	No data
			June 2	17.0	Positive smears	No data
	June 6	Lesions from 5 tuberculous guinea pigs that had been injected with lesions from vaccinated pigs 10 and 11, and lesions from control pig 114.....	June 6	7.4	Positive smears	+
	June 20	Lesions from 4 tuberculous guinea pigs that had been injected with lesions from controls 110 and 111.....	June 20	4.8	Positive smears	+

* Other forms of infection used are described under their respective headings in the text.

† This represents the weight of tuberculous tissue after grinding but before straining through several thicknesses of cheese cloth. The actual amount of original tissue in each dose is less than one-third of this weight.

‡ Made by suspending 10 or 20 grams of thoroughly ground tuberculous tissue in a measured quantity of physiological sodium chloride solution; then estimating according to the technique for the direct microscopic counting of bacteria in milk. The estimate was only comparative. The number of organisms consumed was probably far greater than indicated in this column, since the character of the tissue and the method of estimating did not lend themselves to more definite determination of the number of organisms.

§ The inoculum for the guinea pigs was a portion of the ground and strained tuberculous materials suspended in milk as fed to the calves and the weight is given in terms of ground but unstrained material.

The tuberculous tissues were ground in a meat-grinding machine, weighed after grinding, mixed thoroughly with sufficient warm milk to allow for 100 cc to be fed to each pig, and strained through a single layer of cheese cloth. In practically every case each pig was individually allowed to drink from a pan 100 cc of such mixtures at each feeding. The number of tubercle bacilli in the mixtures naturally varied from time to time. The periods of feeding also varied from daily to intervals of several days. The period of those receiving 10 feedings extended from August 13, 1927, to September 10, 1927; those receiving 20 feedings from September 24, 1927, to October 20, 1927; and those receiving 30 feedings from August 13, 1927, to October 12, 1927.

TABLE 2A
RESULTS OF SUBCUTANEOUS VACCINATION OF SUCKLING PIGS WITH 100 MG BCG AND INFECTION EXPOSURE BY TEN FEEDINGS OF TUBERCULOUS TISSUES
(For controls see table 2B)

Pig No.	Intervals in days			Size of vaccination nodule in mm	Tuberculous lesions*								Guinea pig inoculations	Remarks
	Birth to vaccination	Vaccination to first exposure	First exposure to autopsy		Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver		
1	3	27	90	7 x 7	×	—	×	—	—	—	—	—	D†	Killed because moribund, pneumonia. Liver contained several white areas on surface, 1 mm diameter. Necrotic enteritis. Liver studded with tubercles.
2	3	27	138	15 x 15	×	×	—	—	×	—	×	—	+	Parotid node enlarged 4X, and caseous.
3	3	27	138	10 x 15	×	×	—	—	×	—	×	—	+
4	3	27	162	3 x 5	×	×	—	—	×	—	×	—	+
5	3	27	210	2 x 2	×	×	—	—	×	—	×	—	+
6	5	93	138	5 x 8	×	×	—	—	×	—	×	—	+
7	5	93	138	—	—	—	—	—	—	—	—	+	Left parotid contained caseous foci.
8	5	93	202	?	—	—	—	—	—	—	—	—	+	Lymph nodes that were apparently normal also produced tuberculous in guinea pigs.
9	5	93	238	—	—	×	—	×	—	—	—	+	About 7 months after exposure, became lame in hind leg, followed by posterior paralysis. At autopsy the twelfth thoracic and third lumbar vertebrae were found badly necrosed with tuberculousis.
10	5	93	259	2 x 2	—	—	—	—	×	—	—	—	+	Lesions from submaxillary and ileocecal lymph nodes produced tuberculous in guinea pigs.
11	5	93	259	2 x 2	—	—	—	—	×	—	—	—	+
12	5	93	292	3 x 2	—	—	—	—	×	—	—	—	+
13	5	93	297	?	—	—	—	—	×	—	—	—	+	Left prescapular enlarged 3X, and contained caseocalcareous foci.
14	5	93	297	1 x 1	—	—	—	—	—	—	—	—	+

* The symbols for lesions are adopted from charts used by federal meat inspectors and the names of the various groups and individual lymph nodes or lymph glands correspond in the main to those given by Buckley and Castor. The adjectives bronchial, mesenteric, etc., referring to lymph nodes are, for brevity, used without the words "lymph nodes." Definitions for *slight*, *well marked*, *extensive*, and *no lesions*, have been formulated by the writers as a general guide as follows:

Slight—Caseous or caseocalcareous isolated areas less than 15 mm in diameter.
Well marked—A single caseous or caseocalcareous area, 15 to 50 mm in diameter. Cases having more than one lesion over 25 mm are recorded as extensive.
Extensive—All progressive or multiple miliary tubercles, any one lesion over 50 mm in diameter, or two or more lesions over 25 mm each.
Negative—No lesions found.

† Does not include smears from vaccination lesion.

‡ "D" indicates that guinea pigs died prematurely.

TABLE 2B
RESULTS IN NONVACCINATED PIGS EXPOSED TO INFECTION BY TEN FEEDINGS OF TUBERCULOUS TISSUES

Pig No.	Interval in days		Tuberculous lesions*							Acid-fast bacilli in smears	Guinea pig inoculations†	Remarks
	Birth to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver		
101	30	49	—	—	—	—	—	—	—	—	0	Died; necrotic enteritis present. No vaccinated hog killed at corresponding time.
102	30	112	—	See remarks	—	—	—	—	—	—	0	Died; record of autopsy shows only extensive generalized tuberculosis.
103	30	128	×	×	×	×	×	×	×	×	0	Omentum a mass of tuberculous foci. Died in emaciated condition.
104	30	162	×	×	×	×	×	×	×	×	+	Left precapular lymph nodes enlarged 2X, and caseous.
105	30	210	×	×	×	×	×	×	×	×	+	Right and left inguinals enlarged 2X, and caseous. Costal pleura had 18 to 20 typical nodules 1 to 3 mm in diameter.
106	98	138	—	—	—	—	—	—	—	—	+	Pre-capular lymph nodes contained caseous nodules 3 mm in diameter.
107	98	138	—	—	—	—	—	—	—	—	+
108	98	202	—	—	—	—	—	—	—	—	D	All five guinea pigs injected died within 72 hours.
109	98	238	—	—	—	—	—	—	—	—	+
110	98	257	×	×	×	×	×	×	×	×	+	Many tubercles on parietal pleura, 0.5 to 2.0 cm in diameter.
111	98	257	×	×	×	×	×	×	×	×	+	Lame in right hind leg a few days before killed. Right stifle joint and last lumbar vertebra tuberculous. Precrural and inguinal lymph nodes showed foci.
112	98	292	—	—	—	—	—	—	—	—	+
113	98	292	—	—	—	—	—	—	—	—	+
114	98	297	—	—	—	—	—	—	—	—	+

* For explanation of symbols see footnote of table 2A.

† "D" indicates that guinea pigs died prematurely. "0" indicates that no guinea pigs were injected.

TABLE 3A
RESULTS OF SUBCUTANEOUS VACCINATION OF PIGS WITH 100 MG BCG AND INFECTION EXPOSURE BY TWENTY TO THIRTY FEEDINGS
OF TUBERCULOUS TISSUES
(For controls see table 3B)

Pig No.	Number of feedings	Interval in days			Size of vaccination nodule in mm	Tuberculous lesions*								Acid-fast bacilli in smears†	Guinea pig inoculations	Remarks	
		Birth to vaccination	Vaccination to first exposure	First exposure to autopsy		Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver				Other lesions
15	20	171	18	129	?	×	×	×	×	×	×	—	—	—	+	+	Carpus of right leg enlarged 2X, and tuberculous, Prepectoral lymph nodes caseous, 50 x 40 x 30 mm.
16	20	171	18	132	?										+	+
17	30	148	77	173	?										+	+
18	30	148	77	173	?										+	+
19	30	65	98	173	—										+	+
20	30	85	98	176	15 x 20										+	+
21	30	169	129	134	—										+	+
22	30	158	140	175	?										+	+

* For explanation of symbols see footnote of table 2A.

† Does not include smears from vaccination lesions.

TABLE 3B
RESULTS IN NONVACCINATED PIGS EXPOSED TO INFECTION BY TWENTY TO THIRTY FEEDINGS OF TUBERCULOUS TISSUES

Pig No.	Number of feedings	Interval in days		Tuberculous lesions*								Acid-fast bacilli in smears	Guinea pig inoculation	Remarks
		Birth to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver	Other lesions		
115	20	189	132	×	×	×	×	×	×	×	×	×	+
116	30	225	173	×	×	×	×	×	×	×	×	×	+
117	30	225	173	×	×	×	×	×	×	×	×	×	+	Lungworms present in pneumonic area, 10 x 15 x 15 cm.
118	30	298	134	×	×	×	×	×	×	×	×	×	+

* For explanation of symbols see footnote of table 2A.

Discussion of the Results of Subcutaneous Vaccination and Feeding Exposure.—A general review of tables 2A and 2B, 3A and 3B will disclose the fact that not a single pig was entirely without macroscopic lesions of tuberculosis upon autopsy regardless of age vaccinated, interval between vaccination and exposure, length of exposure, or length of time between exposure and autopsy. It is also evident from a study of the tables that there was no marked difference in the character and extent of the lesions whether or not the pigs received 10, 20, or 30 feedings. Direct comparison of the lesions of vaccinated pigs 1 to 14 and controls 101 to 114 in tables 2A and 2B, with reference to the interval between first exposure and autopsy, favors the vaccinated pigs slightly in the extent and distribution of the lesions. However, if no attention is given to the relation of this time interval the results show no differences in an equal number of vaccinated and controls. It should be noted that all swine represented in this table were of the same age and that the vaccinated were treated at from 3 to 5 days of age and also protected from exposure from 27 to 93 days thereafter. This procedure fulfilled the recommendations of Calmette and Guérin⁽⁵⁾ regarding calves. Tables 3A and 3B represent the results on swine that for the most part were much older when vaccinated and also the group that had a longer exposure period. No significant differences in the extent of tuberculosis could be detected between the vaccinated and the controls although the extent of lesions in the two groups was not as marked as in the younger pigs shown in tables 2A and 2B. Age may have been the factor that limited the amount of infection in this group, since each pig received two or three times the number of feedings of tuberculous material as did those in tables 2A and 2B.

The presence of a vaccination lesion at the site of injection of BCG at the time of, and throughout the exposure period, apparently had no "premunizing"⁵ effect. Observations of the vaccination nodule in pigs Nos. 6–14 (table 2A) were made for the first 72 days, which was 21 days before exposure, and at that time the nodules in the flanks had slightly decreased in size from the maximum size, which occurred between 28 and 42 days. Abscess formation with spontaneous evacuation occurred in only one flank of one pig. The maximum size of the vaccination lesions when measured with calipers through the skin of the flank was approximately 2 × 4 cm. The character of the nodules was hard and nonsensitive. At autopsy, as shown in table 2A, the lesions had either disap-

⁵ According to Calmette, the word "premunition" was first proposed in 1924 by Sergent and Donatien to designate a condition of protective latent infection, such as exists in certain protozoan diseases, particularly bovine piroplasmiasis.

peared or were reduced to a very small size. When incised, a thin connective tissue capsule enclosed a soft caseous pus of slightly greenish white color. Acid-fast organisms were always present in the pus.

INTRAMUSCULAR VACCINATION AND INFECTION EXPOSURES BY FEEDING MILK FROM A TUBERCULOUS UDDER

Swine shown in table 4 were fed milk from an aged tuberculous cow (No. 600) in which tuberculosis of the udder was present. At the time that the feeding tests began on February 5, 1928, the right hind and the left fore quarters were indurated and about twice normal size. The secretion from these quarters was a clear amber fluid, containing a small amount of gray flocculent material which settled quickly on standing. The left fore quarter showed about one acid-fast organism to every five fields. The other quarters were negative for acid-fast organisms by smears. This cow was destroyed on March 6, 1928, which was 4 days after the feeding period closed. Extensive tuberculosis of the lungs, the costal pleura, and the peritoneum, existed together with marked tuberculosis of right hind and left fore quarters. Material from the latter quarter showed only two typical acid-fast organisms after 50 fields were examined and one clump of three acid-fast upon examination of 26 more fields.

During the time that the pigs were being fed milk from cow No. 600 she was being milked night and morning and at each milking gave from 200 to 300 cc of apparently normal milk from the right front teat. From each of the other three teats it was possible to withdraw 20 to 50 cc of abnormal secretion. An occasional microscopic examination of the milk from each of the teats was made during the feeding period and acid-fast bacilli were always demonstrated microscopically in the secretion from the left fore quarter. They were never found in large numbers since it was always necessary to search several fields of a thick smear before any could be found. The entire secretion from the four teats was mixed with a 10 per cent solution of dry skim milk and fed in equal amounts, care being taken to observe that each animal drank the entire portion allotted to it without spilling.

Discussion of Results of Intramuscular Vaccination and Infection Exposure by Milk from Tuberculous Udder.—Reference to table 4 shows two groups of pigs, one of 4 and one of 7, in which very little tuberculosis was produced by the particular type of infection. The groups differ in that the first 4 pigs were just past seven months of age

TABLE 4
RESULTS OF INTRAMUSCULAR VACCINATION* AND INFECTION EXPOSURE BY FEEDING MILK FROM COW WITH TUBERCULOUS UDDER

Pig No.	Amount of BCG vaccine	Interval in days			Size of vaccination nodule in mm	Tuberculous lesions†								Acid-fast bacilli in smear‡	Guinea pig inoculation	Remarks	
		Birth to vaccination	Vaccination to first exposure	First infecting to autopsy		Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver				Other lesions
23	100 mg	186	28	74	50 x 50 x 20	-	-	-	-	-	-	/	-	-	+	-	Right anterior lobe of lung showed glassy nodule 2 mm in diameter. Guinea pig did not develop tuberculosis from injection. Acid-fast bacilli were present in nodule.
24	100 mg	186	28	74	30 x 20 x 20	-	-	-	-	-	-	-	-	-	-	-	No visible lesions. Guinea pigs negative from injection of tissues.
123	Control			74		-	-	-	-	-	-	-	-	-	-	-	No visible lesions. Guinea pigs negative from injection of tissues.
124	Control			74		-	-	-	-	-	-	-	-	-	-	-	No visible lesions. Guinea pigs negative from injection of tissues.
25	100 mg	4	34	74		-	-	-	-	-	-	-	-	-	+	+	Thoracic lymph nodes enlarged 4X to 6X with caseous foci, and about 54 nodules 3-5 mm in diameter, scattered throughout the lungs.
26	100 mg	4	34	74	5 x 5	-	-	-	-	-	-	/	-	-	+	+	Right and left bronchial lymph nodes enlarged and caseous. One nodule 4 mm diameter and 4 nodules 2-3 mm in lungs.
27	100 mg	4	34	74	-	-	-	-	-	-	-	-	-	-	+	+	Guinea pig No. 580 injected with cervical and mesenteric nodes developed generalized tuberculosis.
125	Control			74		-	-	-	-	-	-	/	-	-	+	+	Bronchial lymph nodes enlarged 5X, and caseous. Lungs showed 9 tubercles 2-10 mm in diameter.
126	Control			74		-	-	-	-	-	-	/	-	-	+	+	Right and left bronchial lymph nodes enlarged and 50 per cent caseous.
127	Control			36		-	-	-	-	-	-	-	-	-	+	+	Accidentally killed.
128	Control			36		-	-	-	-	-	-	-	-	-	+	+	Accidentally killed.

* Nov. 23 and 24 received 100 mg BCG in muscles of forearm; Nov. 25, 26, and 27 received 100 mg in muscles of the thigh.

† For explanation of symbols see footnote of table 2A.

‡ Does not include smears from vaccination lesion.

when exposed and the two vaccinated had BCG injected into the muscles of the forearm; whereas the last seven were 38 days old when exposed and the three vaccinated had BCG injected into the muscles of the thigh. Neither controls nor vaccinated acquired tuberculosis in the first group, with the possible exception of No. 23 in which one lobe of the lung showed a 2 mm nodule that contained a few acid-fast bacilli but which did not infect guinea pigs. Since the second group all showed slight macroscopic lesions (with the exception of pig No. 27, whose apparently normal cervical and mesenteric glands, however, produced lesions in one guinea pig), it is to be concluded that (1) infection from the udder was not massive and (2) group one showed greater resistance because of age. It may be further stated that the pigs in the first group were litter mates, and those in group two were litter mates, but the groups came from different sources.

SUBCUTANEOUS VACCINATION AND INFECTION BY INTRAVENOUS INJECTION OF VIRULENT CULTURE

In this group are included 6 vaccinated and 5 controls, all of which were 3½ months old at the beginning of the experiment. Two were given 100 mg of BCG, two, 250 mg, and two, 500 mg, subcutaneously in the flank. Nos. 29 and 33 (table 5), which received 500 mg, were vaccinated by injecting 250 mg into each flank. Sixty days after vaccination the vaccinated and the controls were injected intravenously through an ear vein with 2 mg of bovine tuberculosis culture 271, which had grown upon Duval's medium for 28 days. The history of this culture is as follows: On September 1, 1926, guinea pig 132 was injected intramuscularly with liquid obtained by grinding together tuberculous lesions from 17 cows killed at an abattoir. Guinea pig 132 died of generalized tuberculosis on September 19, 1926, and a portion of its spleen was inoculated into guinea pig No. 210, which died of generalized tuberculosis on November 5, 1926. The spleen of No. 210 was inoculated into guinea pig No. 271, which died of generalized tuberculosis on December 22, 1926. Cultures of tubercle bacilli were obtained from the tissues. These proved to be highly virulent for guinea pigs, rabbits, cattle, and swine.

Discussion of Results of Subcutaneous Vaccination and Intravenous Infection.—Observation of table 5 does not show marked differences in the extent and distribution of tuberculous lesions between vaccinated and control swine though there is some evidence in favor of the vacci-

TABLE 5

RESULTS OF SUBCUTANEOUS VACCINATION AND INFECTION EXPOSURE BY INTRAVENOUS INJECTION OF 2 MG OF VIRULENT CULTURE OF TUBERCULOSIS

Pig No.	Amount of BCG vaccine	Interval in days			Size of vaccination nodule	Tuberculous lesions*							Acid-fast bacilli in smears†	Guinea pig inoculation	Remarks
		Birth to vaccination	Vaccination to injection	Injection to autopsy		Cervical	Bronchial	Mesenteric	Cecal-colic	Transhepatic	Lungs	Spleen	Liver		
28	100 mg	105	60	237	?	/	X	-	-	X	X	/	-	+
29	500 mg	105	60	241	1 cm	-	X	/	-	X	X	/	-	+
30	250 mg	105	60	241	1.5 cm	-	X	/	-	/	X	-	-	+
31	100 mg	105	60	243	3 x 5 mm	-	/	-	-	/	X	/	-	+
32	250 mg	105	60	243	2 x 3 cm	-	X	X	-	X	X	/	-	+
33	500 mg	105	60	243	2 x 1 cm and 4 x 3 cm	/	X	X	-	X	X	/	-	+
129	Control			237			X	-	-	/		/		+	Excellent condition when killed. Only partial intravenous injection in each ear; remainder subcutaneous or intradermal.
130	Control			209		X	X	X	-	X	X	X		+	Killed because of advanced tuberculosis. Costal pleura tuberculous.
131	Control			209		X	X	-	-	X	X	/		+	Killed because of advanced tuberculosis. Partial subcutaneous ear injection; 4 nodules 2 mm in diameter. Costal pleura tuberculous.
132	Control			152		-	X	-	/	X	X	-		+	Killed because of advanced tuberculosis. Costal pleura tuberculous.
133	Control			133		-	-	-	-	-	X	-		0‡	Died and was too decomposed at autopsy for guinea pig injections. Tuberculous pneumonia caused death.

* For explanation of symbols see footnote of table 2A

† Does not include smears from vaccination lesion.

‡ "0" indicates that no guinea pigs were injected.

nated. However, there was a marked difference in the clinical effect upon the two groups. All of the vaccinated were in excellent physical condition when autopsied, whereas 4 of the 5 controls were necessarily killed or had died from emaciation and advanced tuberculosis, in 209 days. On the basis of reported results of experiments by the writers, and by Calmette and Guérin, with calves vaccinated and infected by these methods, greater protection than was obtained was expected in this experiment. The larger doses of BCG did not influence the results. It is evident also that the bovine tuberculosis culture injected intravenously was not as virulent for pigs as for calves. In a previous experiment with 2 control calves, both died at 27 and 32 days respectively, of military tuberculosis, while the first control hog died at 133 days. None of the swine in this group would have been passed for food under official inspection.

INTRAVENOUS VACCINATION AND INFECTION EXPOSURE BY FEEDING

Only 2 vaccinated and 3 controls are reported in this trial. Two other vaccinated and 1 control became sick from an intercurrent disease and their records are not included. Five infecting feedings were given to this group over a period of 12 days, yet these were sufficient to establish moderate lesions in pigs over five months of age when exposed. One mg of BCG was administered intravenously into an ear vein, and 62 days thereafter the first exposure took place.

Discussion of the Results of Intravenous Vaccination and Feeding Infection.—The 2 vaccinated and the 2 controls of the same age, exposed and killed at exactly the same periods, and the 1 control killed only six days later, showed no differences upon which any conclusion as to the effects of this method of vaccination can be drawn, except that no resistance against the infection was evident; in fact vaccinated pig No. 35 had slightly more extensive lesions than any of the controls. Massive infecting doses over a short period of time were used in this group.

INTRADERMAL VACCINATION AND INFECTION EXPOSURE BY FEEDING TUBERCULOUS TISSUES

Two separate groups with 2 vaccinated hogs in each, were included in this experiment (tables 7 and 8). The 2 vaccinated pigs shown in table 7 received 50 mg of BCG in two doses of 25 mg each, 3 days apart. Those in table 8 received the same amount in one dose distributed in three

TABLE 6
RESULTS OF INTRAVENOUS VACCINATION AND INFECTION EXPOSURE BY FIVE FEEDINGS OF TUBERCULOUS TISSUES

Pig No.	Amount of BCG intra-venously	Interval in days			Tuberculous lesions*							Acid-fast bacilli in smears	Guinea pig inoculation	Remarks
		Birth to intra-venous vaccination	Vaccination to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver		
34	1 mg	93	62	165	/	X	/	/	/	/	/	/	+	
35	1 mg	93	62	165	X	X	X	/	X	/	/	/	+	
134	Control	165	X	X	X	/	X	/	/	/	+	
135	Control	165	X	X	X	/	X	/	/	/	+	
136	Control	171	X	X	X	/	X	/	/	/	+	Pancreas contained many foci, 2-5 mm in diameter.

* For explanation of symbols see footnote of table 2A.

different areas. In each case the 50 mg of vaccine was prepared in 5 cc of diluent for injection. The intradermal injections were made along the ventral surface of the abdomen. Exposure of those recorded in table 7 was by means of the ground lesions (prepared as previously described) from control tuberculous hogs, Nos. 112, 113, and 114, and from guinea pigs previously injected with swine lesions. The material for each of the four feedings showed acid-fast bacilli in smears, and guinea pigs injected had generalized tuberculosis when killed. Exposure of those in table 8 was entirely from bovine lesions obtained at a slaughter house, but was not the same as used in some of the other experiments with feeding exposure.

Discussion of Results of Intradermal Vaccination and Feeding Exposure.—The results shown in table 7 are difficult to explain except on the basis that the infecting material was not very virulent. This is indicated by the fact that the 2 vaccinated intradermally, and 4 of the 5 controls, showed no macroscopic lesions; and guinea pigs injected with apparently normal tissues did not develop tuberculosis, except in the case of vaccinated No. 37 whose gastrohepatic lymph nodes produced tuberculosis in two guinea pigs. Tissues from head glands, bronchial lymph glands, and lungs failed to infect guinea pigs. Contradicting the supposition of nonvirulence are the animals exposed to the same material, as shown in table 9: control 140 which developed extensive tuberculosis, and vaccinated pigs 40, 41, 42, 43, and 45 which had mild lesions. The results shown in table 8 are not significant, though the 3 controls had a slightly wider distribution of lesions than did the 2 vaccinated ones.

ORAL VACCINATION AND INFECTION EXPOSURE BY FEEDING TUBERCULOUS TISSUES

In this experiment (see table 9) six pigs received BCG by the mouth. Three were given a total of 3 grams (weight after blotting with filter paper) by administering 1 gram every other day for three feedings. The other 3 were given twice this amount at the same periods. The vaccine was prepared so that each 5 cc. of diluent contained 1 gram. This was then deposited on the fauces by means of a syringe. The first exposure of the 6 treated and the five controls took place 60 days after oral vaccination. All of the pigs were 7 to 8 days of age when the experiment started. The infecting material was the same as that used on pigs in table 7 and as has been stated under discussion of the latter group apparently was not very virulent for the swine.

TABLE 7
RESULTS OF INTRADERMAL VACCINATION AND INFECTION EXPOSURE BY FEEDING TUBERCULOUS TISSUES

Pig No.	Number of feedings	Interval in days				Tuberculous lesion†							Acid-fast bacilli in smears†	Guinea pig inoculations	Remarks
		Amount of BCG intradermally*	Birth to intradermal vaccination	Vaccination to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver		
36	4	50 mg	7	60	200	-	-	-	-	-	-	-	-	-	No lesions except slight verminous pneumonia. Guinea pigs injected with various lymph nodes remained well.
37	4	50 mg	8	60	194	-	-	-	-	-	-	-	-	+	No lesions. Guinea pigs injected with gastrohepatic and mesenteric nodes developed tuberculosis.
140	Control				194	/			-	×	/	×	×	+	The spleen was a mass of tubercles, 1-5 cm. caseous. Liver thickly covered on all surfaces with foci, 2-3 mm.
141	Control				194	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with cervical, bronchial, and mesenteric nodes remained well.
142	Control				186	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various nodes remained well.
143	Control				194	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various nodes remained well.
144	Control				194	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various nodes remained well.

* Pigs received 50 mg in two doses of 25 mg three days apart.

† For explanation of symbols see footnote, table 2A.

‡ Does not include smears from intradermal lesions.

TABLE 8
RESULTS OF INTRADERMAL VACCINATION AND INFECTION EXPOSURE BY FEEDING TUBERCULOUS TISSUES

Pig No.	Number of feedings	Interval in days				Tuberculous lesions†								Acid-fast bacilli in smears‡	Guinea pig inoculations	Remarks
		Amount of BCG intradermally*	Birth to intradermal vaccination	Vaccination to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver			
38	5	50 mg	93	62	165	×	×	×	—	—	—	—	—	—	+	Fibrinous pleurisy present.
39	5	50 mg	93	62	171	×	×	×	—	—	—	—	—	—	+
137	5	Control	165	×	×	×	—	—	—	—	—	—	+
138	5	Control	165	×	×	×	—	—	—	—	—	—	+
139	5	Control	171	×	×	×	—	—	—	—	—	—	+	Pancreas contained many foci, 2-5 mm in diameter.

* Pigs received 50 mg in one dose in three different areas.

† For explanation of symbols see footnote, table 2A.

‡ Does not include smears from intradermal lesions.

TABLE 9
RESULTS OF ORAL VACCINATION AND INFECTION EXPOSURE BY FOUR FEEDINGS OF TUBERCULOUS TISSUES

Pig No.	Total amount of BCG vaccine by mouth *	Interval in days			Tuberculous lesions†							Acid-fast bacilli in smears	Guinea pig inoculations	Remarks
		Birth to oral vaccination	Vaccination to first exposure	First exposure to autopsy	Cervical	Bronchial	Mesenteric	Cecal-colic	Gastrohepatic	Lungs	Spleen	Liver		
40	3 gm	8	60	200	/	-	-	-	-	/	-	-	+	Guinea pigs injected with cervical lesions, and with normal-appearing bronchial and gastrohepatic lymph nodes, developed tuberculosis.
41	3 gm	8	60	200	\	-	-	/	-	-	-	-	+	Guinea pig injected with a cecal caseous focus died of tuberculosis.
42	3 gm	7	60	185	-	-	-	-	-	-	-	-	+	Three pigs injected with other tissues remained normal.
43	6 gm	8	60	200	x	x	-	-	/	?	-	-	+	Lung showed area of pleurisy and hepatization. Guinea pigs injected with bronchial and gastrohepatic lymph nodes developed tuberculosis.
44	6 gm	7	60	185	-	-	-	-	-	-	-	-	-	Cephalic lobe of right lung showed grey hepatization area 8 x 15 cm. Guinea pig died. Three guinea pigs injected with other tissues remained well.
45	6 gm	7	60	200	\	/	-	-	-	/	-	-	+	The guinea pig injected with bronchial lesions developed tuberculosis; the others remained well.
140	Control			104	/	-	-	-	x	-	x	x	+	The spleen was a mass of tubercles, 1.5 cm caseous. Liver thickly covered on all surfaces with foci, 2-3 mm.
141	Control			104	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with cervical, bronchial, and mesenteric lymph nodes, remained well.
142	Control			186	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various lymph nodes remained well.
143	Control			104	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various lymph nodes remained well.
144	Control			104	-	-	-	-	-	-	-	-	-	No lesions. Guinea pigs injected with various lymph nodes remained well.

* Doses of BCG vaccine by mouth were given every other day for 3 days until the total quantity of 3 gm and 6 gm, respectively, was administered.

† For explanation of symbols see footnote, table 2A.

Discussion of Results of Oral Vaccination and Feeding Exposure.—

An interpretation of the data from this experiment is not possible in the light of results on pigs, shown in table 9, which received the same exposure. All the pigs in both groups were from the same source and of the same age, yet 4 out of 5 controls showed no lesions, and only 2 out of 8 that were vaccinated were entirely free of tuberculous changes. If the same principles of interpretation that have been applied to the preceding tables of results are applied to the records in table 9 they would suggest a slightly increased susceptibility following the oral administration of BCG.

OBSERVATIONS OF THE EFFECT OF BCG ON SWINE NOT EXPOSED TO TUBERCULOUS INFECTION

As an additional control on the effects of the vaccine some of the swine treated in various ways with BCG were retained in an environment kept as free as possible from virulent tubercle bacilli. Following is a list of these trials:

1. Fifteen swine at various ages from 1 day to 6 months were vaccinated subcutaneously or intramuscularly with 100-mg doses and held for varying lengths of time from 40 days to 14 months after which they were slaughtered and examined for tuberculous lesions. Small abscesses containing acid-fast bacilli nonpathogenic for guinea pigs were present at the point of injection in most of the swine, but no other evidence of tuberculosis was found. In addition, a boar aged six months was injected subcutaneously and intramuscularly with 80 cc of a suspension containing 800 mg of BCG. The injections were made in small amounts in various parts of the body. On slaughter after 13 months no lesions resembling tuberculosis could be found nor any trace of local lesions at the points of injection.

2. Two pigs, one aged 8 weeks and one aged 7 months, were each fed 7 grams of BCG in milk. On slaughter 6 months later no lesions resembling tuberculosis were found.

3. Eight pigs, aged 6 months, were each injected intravenously with 100 mg BCG suspended in physiological sodium chloride solution. They remained healthy in appearance. Thirty days after injection, 2 were slaughtered and their tissues were apparently normal throughout except that on histological examination the lungs were seen to be studded with lesions attributable to BCG which had lodged in the pulmonary capillaries. Four more were slaughtered 43 days after injection. On macroscopic inspection these were also apparently free from tubercu-

lous lesions but on close scrutiny the lungs were seen to be studded throughout with glass-like nodules, pin point to 0.5 mm in size. A histological study showed these to be minute tubercles containing acid-fast bacilli.

The two other swine were retained for nine months and on slaughter no lesions resembling tuberculosis were found. A close macroscopic scrutiny of the lungs showed nothing abnormal. A histological examination was not made.

In order to test the possibility of producing clinical symptoms by a massive intravenous injection, a gilt aged three months was given by the ear vein 50 cc of physiological sodium chloride solution in which 500 mg of BCG were suspended. The day after the injection, partial paralysis of the rear limbs developed, from which the animal slowly recovered although it was still slightly lame on slaughter 13 weeks after the BCG was injected. At autopsy all tissues were seen to be apparently normal except the lungs which were studded with white nodules up to 0.5 mm in size. A histological study showed these to be necrotic foci containing acid-fast bacilli and surrounded by a wall of epithelioid cells. Guinea pigs inoculated with lung and lymphatic tissue of this gilt remained normal.

The dates on which these trials were made ranged from May, 1926, to September, 1931. The cultures used ranged from the second to the seventieth transplant made following its receipt from Calmette. During this time 328 head of cattle, 356 guinea pigs, and 15 rabbits, as well as the 85 swine mentioned in this paper, were vaccinated. No indication of change in virulence or morphology has been observed in the cultures during these five years. The culture is relatively nonpathogenic for swine. It is capable of causing small lesions at the point of inoculation which soon become surrounded by firm white fibrous connective tissue and are eventually reduced in size or completely disappear.

CONCLUSIONS

One injection of Calmette-Guérin culture (BCG) by subcutaneous, intramuscular, intradermal, or intravenous routes, or three treatments by mouth, failed to give swine sufficient protection against feeding and intravenous infection to prevent generalized tuberculosis.

Slight resistance as compared to the controls was shown by certain groups vaccinated subcutaneously and infected by feeding.

Those vaccinated subcutaneously showed slightly greater resistance against intravenous infection than against feeding exposure, as measured by clinical evidence.

Those vaccinated by mouth showed a slightly greater susceptibility to feeding infection than the control to feeding infection.

Swine over six months of age apparently had more natural resistance to feeding exposure than younger pigs.

No important differences were noted in the extent of tuberculosis produced from 4, 5, 10, 20, and 30 feeding exposures.

The BCG culture used was capable of producing small lesions in swine which eventually healed, at the points where the bacilli lodged after injection. No spread from these primary lesions was observed.

LITERATURE CITED

¹ ASCOLI, A.

1928. *La vaccinazione antitubercolare con bacilli vivi*. 421 p. Istituto Editoriale Cisalpino, Corso Italia 40, Milano.

² ASCOLI, A.

1928. *La vaccination antituberculeuse avec les bacilles vivants*. 235 p. Istituto Editoriale Cisalpino, Corso Italia 40, Milano.

³ CALMETTE, A.

1927. *Techne des cultures de BCG*. *Ann. Inst. Pasteur* 41:201-252.

⁴ DE BLIECK, L.

1930. *Remarks*. Eleventh International Veterinary Congress. Part 1. 225 p. John Bale Sons and Danielsson, Ltd., 83 Great Titchfield St., Oxford St. W. 1. London.

⁵ HARING, C. M., J. TRAUM, F. M. HAYES, and B. S. HENRY.

1930. *Vaccination of calves against tuberculosis with Calmette-Guérin culture, BCG*. *Hilgardia* 4:307-394.

⁶ JERLOV, S.

1926. Cited by Jundell and Magnusson⁽⁷⁾ from *Svensk Veterinärtidskrift* 31:325.

⁷ JUNDELL, I., and H. MAGNUSSON.

1931. *Recherches experimentales relatives a l'action du BCG sur le porc*. *Ann. Inst. Pasteur* 47:408-428.

⁸ SANZ, M. B.

1930. *Vaccination des bovides avec le BCG au Chili*. *Recueil de Méd. Vétér.* 106:136-138.

⁹ CALMETTE, A., and C. GUÉRIN.

1928. *Sur le stade lymphatique de l'infection tuberculeuse chez les bovides*. *Ann. Inst. Pasteur* 42:175-178.

